L25: Entry 19 of 29 File: USPT Apr 9, 1996

DOCUMENT-IDENTIFIER: US 5506127 A
TITLE: Therapeutic grade thrombin produced by chromatography

Brief Summary Text (11):
The invention accordingly provides in good yield a storage-stable therapeutic grade thrombin concentrate of high specific activity and low pyrogenicity, substantially purified with respect to virions. The purification process includes the steps of 1) incubation of crude thrombin starting material with a viricide composition to inactivate lipid-containing enveloped virions; 2) sequential ion exchange chromatography of the incubated material on a single cation exchange medium, using increasing concentrations of phosphate buffer and a sulfalkyl-activated polysaccharide medium such as agarose, dextran, cellulose having a high selective affinity for thrombin, with recovery of a final eluate comprising a phosphate buffer solution of thrombin highly purified with respect to contaminants including other blood proteins, toxins, lipid-containing enveloped virions, and viricide residue; 3) exchange of the phosphate chromatography buffer of the final eluate for a physiologically-compatible formulation buffer to provide a formulation solution of highly purified thrombin; 4) filtration of the thrombin formulation solution over a vital filter to remove non-lipid containing virions; and 5) optional dry heat treatment of filtered and lyophilized thrombin to ensure inactivation of any possible remaining virions (infective vital materials). Aqueous saline formulation buffers of a pH of about 7.2 to 7.4 comprising a citric acid salt and 1) bovine albumin for bovine thrombin intended for veterinary applications, and 2) human albumin for bovine and human thrombin intended for human applications are exemplary. The formulation solutions of the invention (described in more detail below) broadly contribute, inter alia, to stabilization of thrombin enzymatic activity during post-chromatography treatment and during storage; the used formulation also stabilize thrombin against loss of activity during antiviral dry heat treatment according to step (5).

L25: Entry 26 of 29 File: USPT Mar 25, 1986

DOCUMENT-IDENTIFIER: US 4578218 A
TITLE: Purification of factor VIII with insoluble matrix having free sulfate groups covalently bound thereto

Detailed Description Text (16):
A 60.times.1.6 cm siliconised glass column was filled with the dextran sulphate linked Sepharose 4B as in Example 1 and equilibrated at 4.degree. C. at 36 ml per hour with 14 mM trisodium citrate, 2.14 mM calcium chloride pH 6.85. 100 ml blood from a normal donor was collected into 1/10 volume of 3.8% trisodium citrate and platelet-poor plasma obtained following centrifugation at 2000.times.g at 4.degree. C. for 30 minutes. The plasma was made into six separate aliquots of 10 ml in polypropylene tubes and stored overnight at -80.degree. C. Subsequent to thawing at 4.degree. C. and following centrifugation at 4.degree. C. at 2000.times.g for 30 minutes the supernatant was discarded. To the resulting cryoprecipitate in each tube, resuspended in 5 ml of equilibrating buffer at 37.degree. C., was added 1 ml of a (50% vol/vol) suspension of gelatin that had been coupled to Sepharose 4B following the procedure of Cuatrecasas P, Wilchek M and Anfinsen C. B. (1968) Proc. Nat. Acad. Sci. U.S. 61:6367-643, and previously equilibrated with the same buffer. After mixing, the tubes were further incubated at 37.degree. C. for fifteen minutes prior to centrifugation at 3300.times.g for 10 minutes at 37.degree. C. 5 ml of the supernatant from each of the six tubes were removed, combined and applied to the chromatography column. After elution of unbound material was judged to be complete a linear sodium chloride gradient comprising 180 ml initial buffer and 180 ml. 0.8 M sodium chloride was used to develop the bound proteins, collecting 4.0 ml fractions. Factor VIII eluted as a single sharp peak at 0.52 M sodium chloride with a total yield 87% Factor VIIIR:Ag, 85% Factor VIIIR:CoF, 75% Factor VIIIC:Ag and 44% Factor VIII:C. This was completely resolved from all the plasminogen that was shown to elute at 0.15 M NaCl, from the bulk of the fibrinogen that eluted as two distinct components at 0.295 M sodium chloride and 0.375 NaCl, and from the Factor IX which eluted at 0.40 M NaCl. Little fibronectin was found indicating that the initial treatment with gelatin-Sepharose was effective in removing in excess of 98.5% of the fibronectin present in the original cryoprecipitate. Immunoglobulin G (IgG) was detected in many fractions, especially the void material eluting from the column, but less than 1% of the total recovered IgG was found in those fractions containing Factor VIII. Immunoglobulin M was found to elute subsequent to the Factor VIII at 0.62 M sodium chloride. The peak activity of proteolytic enzyme activity measured using the chromogenic substrate S2222 indicated that most of the Factor Xa-like activity eluted coincidentally with the first of the fibrinogen peaks at 0.295 M sodium chloride. The fractions corresponding to Factor VIII contained detectable levels of proteolytic enzyme activity measured with S2222 (Factor Xa) (about 10% of that found in the total eluate from the column) and S2238 (thrombin, about 25% of that found in the total eluate). These proteolytic enzyme activities were not precipitated by treatment at 4.degree. C. with 10% (w/v) polyethylene glygol 6000. This treatment quantitatively precipitated all the Factor VIII. The only other major proteins found to be present in the Factor VIII preparation were various types of lipoproteins. These could be separated from the Factor VIII by ultracentrifugation methods.

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L25: Entry 25 of 29 File: USPT Jul 22, 1986

DOCUMENT-IDENTIFIER: US 4601977 A
TITLE: Method for measuring the activity of plasma factor XIII

Detailed Description Text (14):
20 .mu.l of plasma was sampled and added to 0.1 ml of the buffer solution (1) containing 10% glycerol and the mixture was heated at 56.degree. C. for 4 minutes and then cooled with ice. To this solution were added 0.2 ml of the dansylcadaverine solution, 0.1 ml of the casein solution, 0.05 ml of the calcium chloride solution and 0.05 ml of the thrombin solution in that order and the mixture was reacted at 37.degree. C. for 10 minutes. Immediately thereafter, 0.1 ml of the reaction stopper (6) was added to stop the reaction. 0.5 ml of the resulting solution was poured into the top of the column. When the whole solution has penetrated, 2.5 ml of the buffer solution (1) was added. When the effluent from the bottom of the column has become 3 ml, the fluorescence intensity of this 3 ml effluent was measured in the known way and the activity of XIIIa and the amount of the factor XIII were calculated from the measured value of the fluorescence intensity. The excitation wavelength was 335 nm and the measurement wavelength was 510 nm. The column used in this test was of a cylindrical type of 1.6 cm in diameter. 1 ml of polyvinyl alcohol particles ("Toyopearl HW-40, Coarse", a product of Toyo Soda Co., Ltd.) was used as gel.

L25: Entry 23 of 29 File: USPT Sep 10, 1991

DOCUMENT-IDENTIFIER: US 5047503 A
TITLE: Thrombin-binding substance and process for its preparation

Brief Summary Text (61):
The method (2) may be practised, for example, in the following manner. After dialyzing fresh urine against a buffer having a high salt concentration and approximately neutral pH, active fractions are collected by immune adsorption column chromatography. The active fractions are then subjected to high performance liquid chromatography on an ion-exchange resin, whereby fractions corresponding to the molecular weights of the thrombin-binding substances of this invention are collected to obtain the substances of this invention. As an immunoadsorbent useful for the immune adsorption column chromatography, may be mentioned a monoclonal antibody bound carrier in which a monoclonal antibody for the thrombin-binding substances is bound on an insoluble carrier such as dextran gel, agarose gel or polyvinyl gel. The monoclonal antibody for the thrombin-binding substances may be obtained, for example, by fusing mouse spleen cells, which have been immunized with the above-described thrombin-binding substance extracted from human placentae and having the molecular weight of about 71,000, with mouse myeloma cells P3-Ag8-.gamma. and then treating the resultant hybridomas in a manner known per se in the art. Of monoclonal antibodies available in the above-described manner, it is particularly preferred to use those capable of recognizing sites of the thrombin-binding substances which sites are not affected by calcium. The elution of the thrombin-binding substances from the immune adsorption column can be effected with a buffer containing potassium thiocyanate by way of example.

L25: Entry 22 of 29 File: USPT Jun 2, 1992

DOCUMENT-IDENTIFIER: US 5118790 A
TITLE: Analogs of hirudin

Detailed Description Text (39):
For the clotting assay, hirudin is diluted to 50 ul in assay buffer (50 mM Tris-HCl, 120 mM NaCl, 0.5% PEG-6000, pH 7.4), added to 50 ul of human alpha-thrombin (0.2 pmole) in assay buffer containing 40 mM calcium chloride (final thrombin concentration equal to 1 nM), and incubated for 5 min in a 96-well microtiter plate at room temperature. The reaction is initiated by addition of 100 ul of fibrinogen (10 mg/ml) in assay buffer (without PEG) and the solution mixed for 10 sec. The turbidity of the reaction mixture is monitored at 405 nm using, for example, the Vmax Kinetic Microplate Reader (Molecular Devices Corporation). Data acquisition and processing may be accomplished by a microcomputer interfaced to the microtiter plate reader using software provided by Molecular Devices Corporation.

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L25: Entry 21 of 29 File: USPT May 3, 1994

DOCUMENT-IDENTIFIER: US 5308756 A
TITLE: Protein S chromogenic assay

Detailed Description Text (27):
Microtitre plates were prepared by adding 25 ul of sample, 25 ul of a 0.016M tris-(hydroxymethyl)aminomethane (Tris) buffer at pH 8.0, 25 ul of a starting reagent to each well. The starting reagent was comprised of a water reconstituted lyophilized preparation containing 30 nM activated human Protein C, 30 uM phospholipid, 15 n M activated human Protein C, 30 uM phospholipid, 15 nM calcium chloride, 1.4 uM anti von Willebrands factor, 0.016 M Tris buffer at ph 8, 0.5% polyethylene glycol (PEG) 6000, i% BSA (Bovine Serum Albumin) and 125 mM NaCl. The plates were incubated for 2 minutes at 37.degree. C. to eliminate the effects of the von Willebrands factor. After incubation a 25 ul of a support reagent was added to each well. The support reagent was comprised of a water reconstituted lyophilized preparation containing bovine Factor VIII of at 0.2 units/mL and 0.016 M Tris buffer at pH 8, 0.5% PEG 6000, 1% BSA and 125 mM NaCl and 1.0 mM calcium chloride. The plates were then incubated for 5 minutes at 37.degree. C.. The incubation was followed by addition of 25 ul of a mediator reagent. The mediator reagent was comprised of a water reconstituted lyophilized preparation containing approximately 300 nM bovine Factor IXa, 0.5 uM bovine Factor X, 100 nM bovine Factor IIa and 10 nM (2[N-morpholino]ethane sulfonic acid) (MES) buffer at pH 5.6, 0.5% PEG 6000, 1% BSA, and 125 mM NaCl. The plates were then incubated for 1 minute at 37.degree. C. This incubation was followed by an addition of 100 ul of substrate reagent. The substrate reagent was comprised of a water reconstituted lyophilized preparation containing approximately 0.4 mM CH30-CO-D-CHA-Gly-Arg-pNA, N alpha (2-naphthylsulfonylglycyl) -D-L-Amidinophenyl alaninepiperidide (alpha NAPAP) which is a thrombin inhibitor, 15 mM EDTA, 0.250 M NaCl and 0.020 M Tris at pH 8. The plates were then incubated for 1 minute at 37.degree. C.. This incubation was followed by addition of 50 ul of 1 M citric acid and the spectrophotometric absorbance was obtained at 405 nm using a spectrophotometer.

Detailed Description Text (29):
Semi-micro cuvettes were prepared by adding 50 ul of sample, 50 ul of a 0.016 M tris-(hydroxymethyl)aminomethane (Tris) buffer at pH 8.0, 50 ul of a starting reagent to each well. The starting reagent was comprised of a water reconstituted lyophilized preparation containing 30 nM activated human Protein C, 30 uM phospholipid, I5 mM calcium chloride, 1.4 uM anti von Willebrands factor, .016 M Tris buffer at pH 8, 0.5% polyethylene glycol (PEG) 6000, I% (Bovine Serum Albumin) BSA and 125 mM NaCl. The cuvettes were incubated for 2 minutes at 37.degree. C. to eliminate the effects of the von Willebrands factor. After incubation a 50 ul of a support reagent was added to each well. The support reagent was comprised of a water reconstituted lyophilized preparation containing bovine Factor VIII of at 0.2 units/mL and 0.016 M Tris buffer at pH 8, 0.5% PEG 6000, 1% BSA and 125 mM NaCl and 1.0 mM calcium chloride. The cuvettes were then incubated for 5 minutes at 37.degree. C.. The incubation was followed by addition of 50 ul of a mediator reagent. The mediator reagent was comprised of a water reconstituted lyophilized preparation containing approximately 300 nM bovine Factor IXa, .5 uM bovine Factor X, 100 nM bovine Factor IIa and 10 nM MES buffer at pH 5.6, .5% PEG 6000, 1% BSA, and 125 mM NaCl. The cuvettes were then incubated for 1 minute at 37.degree. C.. This incubation was followed by an addition of 200 ul of substrate reagent. The substrate reagent was comprised of a water reconstituted lyophilized preparation containing approximately 0.4 mM CH.sub.3 O-CO-D-CHA-Gly-Arg-pNA, N alpha (2-naphthylsulfonylglycyl) -D-L-Amidinophenyl

alanine-piperidide (alpha NAPAP) which is a thrombin inhibitor, 15 mM EDTA, 0.250 M and 0.020 M Tris at pH 8. A kinetic determination of the reaction in the cuvette was performed immediately after addition of the substrate by recording the change in optical density at 405 nm for one minute.

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L25: Entry 18 of 29 File: USPT Dec 31, 1996

DOCUMENT-IDENTIFIER: US 5589516 A
TITLE: Liquid preparation of antithrombin-III and stabilizing method therefor

Detailed Description Text (93):
Fifty .mu.l of a dilute sample solution [in 2.4 U/ml heparin, 40 mM Tris-HCl buffer, 0.14M NaCl and 10 mM EDTA (pH 8.4)] was placed in a tube and 100 .mu.l of a human thrombin solution was added [in 0.9% sodium chloride, 0.05% bovine serum albumin (BSA) and 0.05% polyethylene glycol (PEG) 6000; containing 1 U/ml thrombin]. The mixture was preincubated at 37.degree. C. for 5 minutes. Then, 100 .mu.l of a synthetic substrate solution (S-2238: HD-phenylalanyl-L-pipecolyl-L-arginyl-p-nitroanilide dihydrochloride) was added thereto, and the mixture was incubated at 37.degree. C. for 5 minutes. After color development, a citric acid solution (1 ml) was added thereto to stop the reaction, and absorbance at 405 nm was measured with a spectrophotometer. Normal human plasma (1U AT-III/ml) was determined concurrently with the sample determination, and AT-III content in the sample was determined from the calibration curves thereof.

L25: Entry 17 of 29 File: USPT May 6, 1997

DOCUMENT-IDENTIFIER: US 5627038 A
TITLE: Factor IX chromogenic assay

Detailed Description Text (23):
Patient plasma samples were added to a water reconstituted lyophilized preparation containing 4.5 pmol of bovine Factor XIa, 0.2 nmol bovine thrombin, 0.06 nmol calcium chloride, 0.06 umol phospholipids, tris-(hydroxymethyl)-aminomethane (Tris) buffer at pH 8, and stabilizers BSA and polyethylene glycol 6000. Incubation was subsequently carried out alternatively at 25.degree. C., 30.degree. C., and 37.degree. C. for approximately ten minutes.

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L25: Entry 12 of 29 File: USPT Nov 3, 1998

DOCUMENT-IDENTIFIER: US 5831003 A
TITLE: Peptides which bind to prothrombin and thrombin

Detailed Description Text (30):
Human pooled plasma was processed to PTC eluate by standard manufacturing procedures as indicated in Example 1. A sample of the PTC eluate was activated in the presence of 50 mM CaCl.sub.2 for 6 days at 4.degree. C. Approximately 1 ml of the activated PTC eluate, spiked with 300 .mu.g .alpha.-thrombin, was injected onto the peptide resin YFPGPYL-Totda-Toyopearl (FIG. 6). The column was washed with equilibration buffer (10 mM HEPES, pH 6.5, 10 mM CaCl.sub.2, 500 mM NaCl, 0.1% (v/v) PEG-400) until the A.sub.280 returned to base-line. The peptide column was then washed with 1 mM sodium citrate pH 7.0 to release the a-thrombin bound to the peptide. Acid treatment (2% acetic acid) was used as a cleaning step to remove protein still bound to the column.

L25: Entry 9 of 29 File: USPT Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5985833 A
TITLE: Thrombin inhibitor

Detailed Description Text (43):
Thrombin-fibrin binding experiments were performed using a modification of the method reported by Liu, et al. (Liu, C. Y., et al., supra, 1979). Fibrin monomer solutions were prepared from fibrinogen clotted at 1 mg/ml in 60 mM NaH.sub.2 PO.sub.4 buffer, pH 6.4, with thrombin (1 u/ml, final) for two hours at room temperature. The clots were synerized and dissolved in 20 mM acetic acid to >10 mg/ml fibrin, and repolymerized in a 10-fold excess of 100 mM NaCl, 50 mM tris, pH 7.4 buffer containing 40 mM CaCl.sub.2, and 2 mM N-ethylmaleimide. These clots were synerized and dissolved in 20 mM acetic acid to a 10 mg/ml stock solution. Clots containing 0.5 or 1 nmole fibrin were formed by adding a fibrin monomer solution to a 100 mM NaCl, 50 mM HEPES, 0.01% (w/v) PEG 8000, pH 7.4 buffer containing varying amounts of .sup.125 I-labeled PPACK-thrombin, and incubated for 2 hours at room temperature. Clot-bound thrombin was separated from free thrombin by syneresis of the clot. The final concentration of reactants in the clotting mixture were fibrin, 2.5 .mu.M, .sup.125 I PPACK-thrombin, 0 to 37.5 .mu.M, in a final volume of 200 or 400 .mu.l. For clotting mixtures containing des B.beta.1-42 fibrin, which polymerizes slowly and incompletely, full clot recovery (>95%) was assured by crosslinking the fibrin with factor XIIIa (25 u/ml) for 2 hours at room temperature. After the incubation period, tubes were centrifuged and thrombin-bound clots separated from free thrombin by syneresis. The distribution of thrombin bound to the clot and free in solution was determined by radioactivity counting in a Packard Multi-prias 3 gamma counter. The amount of thrombin trapped in the clot was estimated from the radioactive counts that were retained in crosslinked clots of peak 1 or des B.beta.1-42 peak 1 fibrin in the presence of 25 pM S-Hir.sup.53-64, which had been added to block thrombin exosite binding to fibrin.

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L25: Entry 29 of 29 File: DWPI Jan 26, 1993

DERWENT-ACC-NO: 1993-071038
DERWENT-WEEK: 199309

TITLE: Blood vessel blocking agent to treat brain aneurysm and tumour - contains thrombin, polyethylene glycol! and heavy metal e.g. gold, silver, bismuth or thorium

PATENT-ASSIGNEE:

ASSIGNEE	CODE
TERUMO CORP	TERU

PRIORITY-DATA: 1991JP-0193630 (July 9, 1991)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 05017369 A	January 26, 1993		003	A61K037/54

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP05017369A	July 9, 1991	1991JP-0193630	

INT-CL (IPC): A61K 37/54; A61K 49/04

ABSTRACTED-PUB-NO: JP05017369A
BASIC-ABSTRACT:

The agent comprises an aq. suspension contg. thrombin, heavy metal, and polyethylene glycol.

The heavy metal is Au, Ag, Bi or Th. Polyethylene glycol has mol.wt. 10,000-100,000. 0.01-50% (w/v) of heavy metal is boiled, mixed with 0.1-5.0% of citrate, cooled with 0.1-10% of polyethylene glycol, and controlled with acetic acid for pH 5.9 to form colloid. Thrombin and polyethylene glycol are added to the colloid, and centrifuged to obtain a ppte., which is mixed with phosphoric acid buffer contg. polyethylene glycol with thrombin. The blockage contains 0.1 u/ml-200 u/ml of thrombin, 0.01-50% of heavy metal and 0.1-10% of polyethylene glycol.

USE/ADVANTAGE - The block has clear contrast and is useful for therapy of brain aneurysm, tumour, et

CHOSEN-DRAWING: Dwg.0/0

TITLE-TERMS: BLOOD VESSEL BLOCK AGENT TREAT BRAIN ANEURYSM TUMOUR CONTAIN THROMBIN POLYETHYLENE POLYGLYCOL HEAVY METAL GOLD SILVER BISMUTH THORIUM

DERWENT-CLASS: A96 B04 B06

CPI-CODES: A05-H03A; A12-V01; B04-B04D3; B04-C03C; B05-A03B; B12-G07; B12-H04;

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L25: Entry 28 of 29 File: JPAB Jan 26, 1993

PUB-NO: JP405017369A
DOCUMENT-IDENTIFIER: JP 05017369 A
TITLE: THROMBOEMBOLIC AGENT

PUBN-DATE: January 26, 1993

INVENTOR-INFORMATION:
NAME COUNTRY
EZAKI, YUZO
ISHIYAMA, HARUO
SAWAMOTO, JIRO
HASHIMOTO, CHIKAO

ASSIGNEE-INFORMATION:
NAME COUNTRY
TERUMO CORP

APPL-NO: JP03193630
APPL-DATE: July 9, 1991

INT-CL (IPC): A61K 37/54; A61K 49/04

ABSTRACT:

PURPOSE: To obtain a thromboembolic agent consisting of an aqueous suspension containing thrombin, heavy metal and polyethylene glycol and useful for treatment for aneurysm, vascular malformation, hemostasis and tumor.

CONSTITUTION: The objective thromboembolic agent consisting of an aqueous suspension containing thrombin, heavy metal (e.g. gold, silver, thorium, etc.) and polyethylene glycol (preferably having 10000-100000 molecular weight). The agent is prepared by e.g. boiling 0.01-50% tetrachloroauric (III) acid, adding 0.1-5.0% citric acid thereto, quickly mixing these ingredients and cooling the mixture to ambient temperature, adding 0.1-10% polyethylene glycol (PEG) thereto, controlling pH of the mixture to 5.9 by acetic acid to prepare gold colloid, adding thrombin and PEG to the gold colloid, centrifuging the mixture, re-floating the resultant precipitate in phosphoric acid buffer solution (having pH7.2) and adding thrombin thereto.

L25: Entry 27 of 29 File: JPAB Nov 28, 1995

PUB-NO: JP407308190A
DOCUMENT-IDENTIFIER: JP 07308190 A
TITLE: PRODUCTION OF THROMBIN

PUBN-DATE: November 28, 1995

INVENTOR-INFORMATION:
NAME COUNTRY
HANADA, SHINICHI
HONDA, KEISHIN
MORISADA, YASUAKI
MIYAKE, SHOICHI
MATSUMOTO, TAKEHIKO

ASSIGNEE-INFORMATION:
NAME COUNTRY
GREEN CROSS CORP:THE

APPL-NO: JP06127094
APPL-DATE: May 18, 1994

INT-CL (IPC): C12 N 9/74; A61 K 38/46

ABSTRACT:

PURPOSE: To obtain a thrombin having coagulating action from an inexpensive material industrially and advantageously, by treating a prothrombin-containing aqueous solution with a Ca salt in the absence of thromboplastin and plasma.

CONSTITUTION: The supernatant after a cryoprecipitate is removed from human plasma is brought into contact with an anion exchanger such as DEAE-dextran. An adsorbed prothrombin complex is eluted to give a prothrombin-containing aqueous solution. Then the prothrombin-containing aqueous solution is treated with 3% aqueous solution of calcium chloride at 0-15°C in the absence of thromboplastin and plasma for three days, adjusted to pH 7, treated with tri-N- butyl phosphate, ε-aminocaproic acid, etc., then brought into contact with an cation exchanger such as sulfopropyl-dextran and an adsorbed substance is eluted to give a thrombin having coagulating action by using an inexpensive material on an industrial scale.

L25: Entry 24 of 29 File: USPT Mar 12, 1991

DOCUMENT-IDENTIFIER: US 4999194 A
TITLE: Two-chain urokinase plasminogen activators for treatment of thrombotic disease

Detailed Description Text (15):
To confirm that cleavage by thrombin has gone to completion the reaction mixture is analyzed by two methods. (1) an aliquot is reduced with dithiothreitol and analyzed by SDS polyacrylamide gel electrophoresis (Laemmli, U. K., Nature 227, 680, 1970) and silver staining (Heukeshoven, J. and Dernick, R., Electrophoresis 6, 103-112, 1985). This demonstrates that scu-PA is converted completely to a two chain molecule by thrombin. (2) The amidolytic activity of the thrombin treated scu-PA is quantitated in a 1 ml reaction containing 0.1 ml of thrombin treated scu-PA, 0.1 ml of 3 mM S-2444 (pyro GLU-GLY-ARG-para-nitroanilide; Kabi Vitrum, Stockholm, Sweden), 0.8 ml of 50 mM Tris-HCl (pH 8.8), and 38 mM NaCl and 0.1% polyethylene glycol. The reaction mixtures are incubated at 37 degrees C. for 5 minutes, then stopped by the addition of 0.1 ml of 50% acetic acid, and the absorbance at 405 nm determined. Amidolytic acitivity of the sample is calculated based on the standard curve generated using urinary urokinase as a standard. The amidolytic activity of the thrombin-cleaved scu-PA is less than 1000 IU/mg (protein concentration determined using the method of Lowry).

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L23	l22 same l18	1906	L23
L22	l15 or l16	619521	L22
L21	l17 same l18	63	L21
L20	buffer or hepes or mops or tris	731145	L20
L19	pva or polyvinyl alcohol or peg or polyethylene glycol or pvp or polyvinyl pyrrolidone or dextran or ficol	298609	L19
L18	thrombin	13505	L18
L17	l15 same l16	26036	L17
L16	formic acid or acetic acid or propionic acid or butyric acid or valeric acid or lactic acid or malic acid or citric acid or organic acid	355145	L16
L15	calcium	370616	L15
L14	l10 and l11 and l12 and l13	1	L14
L13	triacetin	3526	L13
L12	polyethylene glycol	125392	L12
L11	silica$5	473308	L11
L10	COX-2 inhibitor$5	666	L10
L9	L8 not l4	2	L9
L8	l6 and l1	10	L8
L7	l6 same l1	1	L7
L6	glucagon-like peptide-1	232	L6
L5	glucagon like-1 peptide	0	L5
L4	l1 and l2	17	L4
L3	l1 same l2	1	L3
L2	GLP-1	489	L2
L1	cardiomyopath$6	2716	L1

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